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Bacteriological, mineral and radioactive contents of leachate samples from dumpsite of Ekiti State Government Destitute Centre in Ado-Ekiti

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ABSTRACT

The leachate samples from the Ekiti-State Government Destitute Centre used by the Ekiti State Waste Management Board [EKWMB] Ado-Ekiti were subjected to bacteriological, mineral and radioactive analyses. The bacteria were isolated using serial dilution procedure and pour plate method. Biochemical tests like catalase, citrate and so on were used to identify the isolated organisms. Atomic Absorption Spectrophotometry method was used to analyze the heavy metal contents in the leachate samples. Natural radioactivity content of soil samples from the dumpsite was also determined using a NaI(Tl)-based gamma counting system. The total bacteria and coliform counts ranged from 70.6 x 10^7 CFU/ml to 7.3 x 10^8 CFU/ml and 39.9 x 10^7 CFU/ml to 1.9 x 10^8 CFU/ml respectively. There is significant difference at $P<0.05$ between total bacteria and coliform count base on period of sample collection whereas there is no significance between total bacteria and coliform based on points of collection. The isolated bacteria and percentage occurrence includes; Escherichia spp (32%), Enterobacter spp (18%), Klebsiella spp (14%), Bacillus spp (12%), Enterococcus spp (9%), Salmonella spp (6%), Pseudomonas spp and Staphylococcus spp (4%). The mineral value ranged as follow; zinc (0.001mg/L–0.02mg/L), lead (0.001mg/L-0.002mg/L), copper (0.001mg/L–0.02mg/L), cobalt (0.001mg/L- 0.02mg/L) and in all samples mercury was not detected. Mean concentrations of 974±67, 35±3 and10±2 Bq kg $^{-1}$ were obtained for ^{40}K, ^{226}Ra (^{238}U) and ^{208}Tl (^{232}Th) respectively. The average absorbed dose rate and annual effective dose equivalent amounted to 63nGy h^{-1} and 0.08 mSv y^{-1} respectively. These values did not constitute any radiological burden to human population. All the organisms exhibited a high level of resistance to most of the antibiotics used. There is urgent need for awareness to be created about the present situation of the leachate to alert the communities living around the area on the need for treatment of the stream before they can be used for drinking and other domestic uses.

Key words: Coliform, total bacteria, radiological.

INTRODUCTION

Excessive human consumption and the management of generated solid waste are complex problems that continually confront the society. Generally, these wastes are dealt with in the simplest and least expensive way, by accumulating the waste in uncontrolled dumping sites (UDS) without any concern as to their leaching and transport fate into groundwaters. Owing to global development and the consumerism attitude, the quantity of waste increased considerably, and UDS have become a real danger to the environment and public health. For these reasons, UDS are being replaced by controlled sanitary landfills [1] which are aimed at treating waste in a more sustainable way.

Landfills are the most widely used solid waste disposal method across the world. Studies on landfills have been mainly devoted to waste composition, gas emission and physical parameters. Despite the importance of microorganisms in the decomposition of organic matter, knowledge on the bacterial population is still fragmentary. Waste management has become increasingly complex due to the increase in human population, industrial and technological revolutions and the processes that control the fate of wastes in the soil is complex and many of them are poorly understood. Issues such as nutrients release rate and other chemicals, leaching of nutrients, metals through macro pores as suspended solids and sludge organic matter on the sorption degradation are often not understood by many [2].

The microbiology of landfill ecosystems has not been thoroughly explored; they are unique anaerobic ecosystems with abundance of degradable organic carbon, and a wide range of microbial activities due to its heterogeneous composition [3].

Landfills contain large numbers of pathogenic and opportunistic bacteria, due to the presence of used disposable napkins and sanitary towels, clinical waste and domestic human origin waste as hypodermic needles and syringes [4, 5]. Studies about pathogenic and opportunistic bacteria in landfills are scarce. In a review published in 1992, there were 16 pathogenic species listed, the most important of them were: *Acinetobacter calcoaceticus, Enterobacter cloacae,* some serotypes of *Escherichia coli, Klebsiella pneumoniae, Listeria monocytogenes, Proteus spp, Pseudomonas aeruginosa, Salmonella spp, Serratia marcescens, Staphylococcus aureus* and *Yersinia enterocolitica* [6]. [7] isolated 13 pathogenic and opportunistic bacteria from samples of a domestic waste transfer station in Mexico City; *Citrobacter, Enterobacter, Escherichia, Hafnia, Klebsiella, Salmonella, Serratia* and *Yersinia* species were identified in several samples.
Leachate is the liquid generated from moisture associated with materials within the landfill cell, after field capacity has been reached. Its production may be thought of as landfill percolation. Although the rate of production of leachate over a given year is influenced by the volume of rainfall percolation through the landfill, among other factors, it is quite likely that there would be a significant volume build-up if provision is not made for its continual removal. A myriad of volatile organic molecules concentrate in the solution. By virtue of their complexity, their resistance to biodegradation, and their quantities, some of the accumulated molecules render the ever concentrating leachate solution highly toxic. The actual composition of leachate solution may vary greatly within individual landfills over time, as well as among different landfills largely because of the chemical composition of the waste itself, but also because of the amount of precipitation in the area and other site-specific conditions. Some of the different chemicals

contained in leachate have been categorised as Volatile Organic Compounds, Metals/metal ions, Synthetic Organic Compounds, Other.

Man and his environment are constantly being exposed to ionization. It can be either natural in its origin (Primordial and Cosmogenic) or artificial (Anthropogenic) which are due to human activities such as generation of electricity, oil and gas production, experimental laboratory research, mining activities, use of x-ray in medicine, painting industry and agricultural activities. The waste dumpsites are liable recipient of any radioactive materials and waste from above uses. Domestic and industrial wastes contain various substances which include radioactive materials resulting from the use of processing chemicals. Also remnants from staple foods contain traces of radioactive materials or contaminants. The disposal of these wastes in dumps without adequate management exposes the population to radiation hazard. This is because people that live around, do use waste for compost and build on waste dumpsites without considering the radiological implication [8].

The aim of this research work was to collect and analyze bacteriologically the leachate samples from Ekiti State destitute centre in Ado-Ekiti, the antibiotics sensitivity/susceptibility of the isolated bacteria will be determined. Also the heavy metals in the leachate samples will be determined. The need to obtain adequate knowledge of radiation exposure in soil from waste dumpsites in the city informed this present investigation.

MATERIALS AND METHODS

Description of the study area and sampling station

The study area is the Ekiti State Government Destitute Centre used by the Ekiti State Waste Management Board [EKWMB]. Most of the wastes disposed are mainly domestic and household wastes. Domestic wastes is from food residues from kitchen, hair and dead skin cells from bath/shower water and human excreta (urine and faeces) while Household waste include everything from lawn clippings to burned out light bulbs.

Collection of samples and bacteriological analysis

The leachate samples were collected aseptically with the aid of a sterile needle and syringe at different sampling points. Each of the samples were collected in separate well labeled sterile bottle and placed in an ice bag properly and transported to the laboratory. A total of fifty samples were collected.

For each sample 1 ml of leachate was diluted on 10 ml of PBS and vortexed vigorously. With this suspension, diluted series to extinction were prepared with phosphate buffer pH 7.4 (1:10-1:10-8), by triplicate. The last dilution of every series was grown on general and selective agar media. MacConkey Agar for selective isolation of *Salmonella typhi*, *Proteus* or *Pseudomonas* species and *Salmonella-Shigella A*gar.1 incubated at 37^{O}C during 48-76 h in aerobic condition. The pure bacterial strains were identified on the basis of their morphological and biochemical tests. The pure cultures of the bacterial isolates were subjected to various morphological and biochemical characterization tests such as color, shape, elevation, consistency , margin , Catalase test, MRVP (methyl red-voges proskauer test), fermentation of sugars, kovacs citrate, indole, hydrolysis of starch, and sensitivity tests. In order to determine the identity of bacteria isolates,

results were compared with standard references of Bergey's Manual of Determinative Bacteriology 2nd edition [9, 10].

Antibiotic susceptibility test

The antibiotics susceptibility of the isolates was determined by the disk diffusion method on Mueller-Hilton agar according to [11]. The bacterial isolates were tested against seven ABTEK disc antibiotics which comprised Cefotaxime (CAZ 30µg), Cefuroxime (CRX 30µg), Gentamycin (GEN 10µG), Ofloxacin (OFL 5µg), Augmentin (AUG 30µg). Gram negative disc contains additional constituent such as Nitrofurantoin (NIT 300µg), Ceftazidme (CTX 30µg) and Amoxicillin (AMX 30 µg). Gram positive disc contains additional constituent such as Lincomycin (LIN 2µg), Oxacilin (OXA 10µg) and Cloxacilin (COX 5µg). The inoculum was standardized by adjusting its density to equal the turbidity of a barium sulphate (BaSO4) (0.5 McFarland turbidity standard), and incubated at 35oC for 18 hours. The diameter of the zone of clearance (including the diameter of the disk) was measured to the nearest whole millimeter and interpreted on the basis of CLSI guideline [11].

Determination of the heavy metals

$5cm^3$ of concentrated HNO_3 was added to $200cm^3$ of the leachate sample in $250cm^3$ beaker. The solution was evaporated near dryness on a hot plate. After cooling, another $5cm^3$ concentrated HNO_3 was added and the beaker was covered with a washed glass. Gentle heating was then continued until digestion was completed. Additional $10cm^3$ of concentrated HNO_3 was then added and the content filtered was made to $5cm^3$ with distilled water. This was then subjected to Atomic Absorption spectrophotometer (AAS) and the reading was taken in mg/L.

Collection and preparation of soil samples for radioactive analysis

Five (5) sampling locations of dimension 0.5 m x 0.5 m was selected around the waste dumpsite WDS. Soil samples were collected at the centre of each sampling location using coring tools. Utmost care was taken in the extraction of soil sections to avoid mixing or cross contamination of soil samples.

For low level radioactive sources such as soils, the gamma radiation intensity is low except where a large mass of the sample is analyzed. But there is a limit to how much mass can be placed in the counting system. As a result of this, the samples were prepared into forms that will enhance the detection of the gamma ray intensity. To accomplish this, individual soil sample was thoroughly dried at room temperature to constant weight and sundry at 25 ± 2^0C to drain off water. The samples were also oven dried at a temperature of 105^0C [12]. The removal of moisture took care of self-absorption in each of the samples. The dried samples were pulverized into fine grains so as to increase the total emission area [13]. They were then packed in two hundred and fifty (250 g) by mass and sealed in gas-tight, radon impermeable trap-shape hermetically sealed plastic containers whose diameter are of the same dimension with the diameter (7.6 cm) of the detector surface. The sealed samples were kept for a minimum of 30 days before counting to re-establish the radioactive equilibrium between the series radionuclides and their daughter products due to the possible escape of radon gas during handling [14, 15].

After preparation of samples had attained secular equilibrium, each sample was placed directly on the detector for counting. The counting time for each sample was 36000 s (10 hrs). This time

was chosen for greater accuracy which demands longer time especially when the radioactive content is low like the environmental samples in this study. Gamma counting of the samples was performed on a low level gamma ray spectrometer consisting of a 7.6 cm x 7.6 cm NaI (Tl) detector directly coupled to a pre-amplifier, a computer-controlled multichannel analyzer (MCA) which consists of a hardware Analogue to Digital Converter (ADC) that sorts the input pulses into 512 different pulse amplitude channels and a software controlling the hardware and storing the data accumulated. The radium content of the samples was determined from the intensity of the 1.765 MeV peak of ^{214}Bi, the Thorium activity was determined from the 2.615 MeV gamma ray peak of ^{208}Tl(^{232}Th) and Potassium activity was determined using 1.460 MeV decay of ^{40}K.

Statistical analysis

The results are expressed as Mean ±SD. Difference in means were also determined by Duncan's multiple range test ($P<0.05$).

RESULTS AND DISCUSSION

The total bacterial counts in the first week of collection of sample have a mean ranged between 17.6 x 10^7 CFU/ml and 7.3 x 10^8 CFU/ml. The second week of collection of samples have a mean ranged between 19.5 x 10^7 CFU/ml and 12.1 x 10^8 CFU/ml. the mean of the total bacterial count obtained in third week ranged between 40 x 10^7 CFU/ml and 26.5 x 10^8 CFU/ml. The mean of total bacterial counts obtained in the fourth week ranged between 33 x 10^7 CFU/ml and 21 x 10^8 CFU/ml and the mean value of the total bacterial counts in the fifth week ranged between 70.6 x 10^7 CFU/ml and 33.3 x 10^8 CFU/ml (Table 1). Table 2 shows the mean total coliform counts obtained in the first week of collection ranged between 10.7 x 10^7 CFU/ml and 4.3 x 10^8 CFU/ml while mean total coliform counts obtained in the second week ranged between 3.7 x 10^7 CFU/ml and 1.9 x 10^8 CFU/ml. The mean value of coliform count obtained in the third week ranged between 5.5 x 10^7 CFU/ml and 2.5 x 10^8 CFU/ml. The mean total coliform count in the fourth week ranged between 9.2 x 10^7 CFU/ml and 6.5 x 10^8 CFU/ml, while in the fifth week mean total coliform count ranged between 39.9 x 10^7 CFU/ml and 21.7 x 10^8 CFU/ml.

The bacterial percentage of occurrence revealed that frequency was highest for *Escherichia coli* with 32% followed by *Enterobacter spp* with 18% of occurrence, *Klebsiella* spp (14%), *Bacillus spp (*12%), *Enterococcus* spp (10%), *Salmonella spp* (6%), while *Pseudomonas spp* and *Staphylococcus aureus* occur at minimal percentage of 4% each (Table 3). The bacteria isolated include species known to be involved in the degradation of organic matter. Among the bacteria isolated, *Escherichia spp* has the highest percentage of occurrence while *Pseudomonas spp* and *Staphylococcus aureus* has the least percentage of occurrence, This complies with the report of [16] which stated that *Escherichia coli* is able to withstand competition from other indigenous organisms with high growth rates. The presence of *Escherichia coli* is chiefly due to fecal contamination and it is an indication of the likely presence of other pathogenic bacteria which are capable of causing serious diseases. All the bacteria isolated during this investigation have been reported by [17] as potential pathogens. The presence of these potential pathogens in the leachate may be attributed to the disposal of raw human faecal discharges and other human wastes at the waste-dump site of the leachate. [3] reported that there is a re-growth of enteric bacteria in the cooler exterior of the dump so that populations of pathogenic organisms continue

to survive and also that truly pathogenic forms of microorganism may survive in waste or leachate.

From the result of the antibiotics sensitivity test, it was noted that there is widespread of resistance to antibiotics among the bacteria isolated from the leachate. Nearly all the organisms were resistant to most of the antibiotics for which they were tested against. The organisms may become resistant due to production of enzymes which inactivates or modify antibiotics, changes in bacterial cell membrane, modification of target site, and development of metabolic pathways by bacteria. These properties are acquired when bacteria undergo genetic changes. Such a genetic change may occur by mutation or by acquisition of new genetic material [17]. Selection of resistant organisms in nature may result from natural production of antibiotic by soil microorganisms, runoff from animal feed, crops or waste product from treated livestock or humans [18]. The passage of leachate through the topsoil enables it to acquire soil microorganisms as such; these organisms have derived means to detoxify the effects of these antibiotics thus having little or no effects on them. [19] reported that the transfer of antibiotics resistance gene from one organism to another is a reason for the high antibiotics resistance pattern in these organisms.

When leachate is discharged into the water bodies, individuals drinking this untreated water or using it for other domestic purposes may ingest the resistant strain and these strains will become part of the human microflora; as a result of selection pressure, such organisms may establish themselves within the individuals and become predominant microflora. Therefore, infections caused by such organisms are very difficult to treat [18].

The result of the heavy metals analysis as shown in Table 5, indicated that elements such as zinc, lead, copper and cobalt where present in a very minimal amount. copper and cobalt has the highest concentration values ranging from 0.001mg/L to 0.02mg/L while zinc and lead has the least concentration values ranging from 0.001mg/L to 0.002mg/L. Mercury was not detected in any of the leachate samples, This complies with the work of [20] who reported that heavy metals are poisonous to microorganisms and because they are present in minute amount, gives the reason for the increase in growth of the microorganisms as shown in Table 1 and 2. It can be deduced from this research that the lower the heavy metal content in the leachate, the higher the microbial load of the leachate.

The result of the radioactivity in Table 6 showed that net area count after background corrections in each photopeak was used in computation of the activity concentration of each of the radionuclides using the expression.

$$C_c(Bqkg^{-1}) = \frac{A_{net}}{E\gamma tm}$$

Where E is the detection efficiency, A_{net} is the net area under the peak, t is the counting time and γ is the gamma yield, that is the fraction of the γ-rays of the particular energy per disintegration and m is the mass of the sample [21, 22].

The mean activity concentrations calculated for each radionuclide in the location using equation above are 974 ± 67, 35 ± 3, 10 ± 2 Bq kg^{-1} for ^{40}K, ^{226}Ra(^{238}U) and ^{208}Tl(^{232}Th) respectively.

Artificial radionuclides such as ^{137}Cs were not detected in any of the samples analysed. The activity concentrations of ^{40}K were the highest compared with those of other radionuclides for all the sampling locations. This is in agreement with the trend common to natural environmental radioactivity [23].

These activity concentrations were only indication of levels of radionuclides present and do not relate the effect of such level on bio-system especially when these soils are used for agricultural purposes and when building are erected on the site. The important quantity to assess when considering radiation risk to a bio-system is the absorbed dose rate. The absorbed outdoor dose rate, D_{out} (nGy h^{-1}) in air at 1m above the ground level due to the concentrations of radionuclides in the samples is calculated using a relation presented below [24, 25, 26, 8, 22]:

$$D_{out} = a.C_{Ra} + b.C_{Th} + _{C}.C_{K} + d.C_{CS}$$

where a is the dose rate per unit ^{226}Ra activity concentration (4.27x10^{-10} Gy.h^{-1}per Bq.kg^{-1}), C_{Ra} is the concentration of ^{226}Ra in the sample (Bq.kg^{-1}), b is the dose rate per unit ^{228}Th activity concentration (6.66x10^{-10} Gy.h^{-1} per Bq.kg^{-1}), C_{Th} is the concentration of ^{228}Th in the sample (Bq.kg^{-1}), c is the dose rate per unit ^{40}K activity concentration (0.43x10^{-10}Gy.h^{-1}per Bq.kg^{-1}), C_K is the concentration of ^{40}K in the sample (Bq.kg^{-1}), d is the dose rate per unit $^{137}C_s$ activity concentration (0.03x10^{-10} Gy.h^{-1} per Bq.kg^{-1}), and C_{Cs} is the concentration of ^{137}Cs in the sample (Bq.kg^{-1}). Since ^{137}Cs were not detected in any of the samples, the last term in Equation was taken as zero. The mean absorbed dose rate for the site is 63 ± 9 nGy h^{-1}. The quantity absorbed dose is a very useful physical concept; but in biological systems the same degree of damage is not necessarily produced by the same absorbed dose of different types of radiation in a given organ.

Applying the conversion factor of 0.7 Sv.Gy^{-1}, which converts absorbed dose in air to human effective dose and using an outdoor occupancy factor of 0.2 as recommended by [24], the average annual effective dose due to gamma-radiation from these terrestrial sources at the waste site was assessed. However, since people because of socio-economic reasons would always be in the city, 0.2 outdoor occupancy factor was used in this study to adequately describe the scenario being considered. Assuming this, the average outdoor effective dose was therefore calculated using equation below:

E_{air} = D (nGyh^{-1}) x 8760 (hy^{-1}) x 0.2 x 0.7 (Svy^{-1}) x 10^{-6}
This equation can be summarized as:
$E_{air} = TQD_{air}\boldsymbol{\varepsilon}$

Where E_{air} is the annual effective dose rate in (mSv.y^{-1}), T is time, (8760 h.y^{-1}), Q is the quotient of the effective dose and absorbed dose rate in air (0.7 Sv.Gy^{-1}), $\boldsymbol{\varepsilon}$ is a factor converting nano (10^{-9}) into milli (10^{-3}) and D_{air} is the absorbed dose rate in air (nGy.h^{-1}). The calculated annual effective dose is 0.08 mSv.y^{-1} . This value is much less than the recommended dose limit of 1 mSv.y^{-1} for the members of the public [24, 27]. Therefore, it suffices to say that no radiological burden is envisaged when the site is being put to various uses.

Table 1: Total Bacterial Count of the leachate sample (CFU/ml)

Sampling point	Dilution Factor	
	10^7	10^8
A_1	50	21
A_2	17	3
A_3	5	2
A_4	20	17
A_5	14	7
A_6	2	0
A_7	63	23
A_8	2	0
A_9	1	0
A_{10}	2	0
Mean Value	**17.6**	**7.3**
B_1	6	2
B_2	10	8
B_3	49	20
B_4	30	25
B_5	7	3
B_6	56	39
B_7	8	5
B_8	4	2
B_9	4	1
B_{10}	21	16
Mean Value	**19.5**	**12.1**
C_1	24	17
C_2	15	7
C_3	39	20
C_{4s}	44	30
C_5	14	3
C_6	42	29
C_7	47	30
C_8	45	34
C_9	45	31
C_{10}	85	64
Mean Value	**40.0**	**26.5**
D_1	18	10
D_2	15	10
D_3	20	16
D_4	27	17
D_5	25	15
D_6	12	8
D_7	40	20
D_8	44	30
D_9	100	74
D_{10}	29	10
Mean Value	**33.0**	**21.0**
E_1	42	31
E_2	24	16
E_3	44	11
E_4	13	7
E_5	51	42
E_6	80	20
E_7	84	30

E_8	96	42
E_9	150	52
E_{10}	122	82
Mean Value	**70.6**	**33.3**

A, B, C, D and E - period of collection of sample (Days)
1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 -point of sample collection.

Table 2: Total Coliform Count of the leachate sample (CFU/ml)

Sampling point	Dilution Factor	
	10^7	10^8
A_1	7	3
A_2	3	2
A_3	3	1
A_4	8	4
A_5	3	0
A_6	6	1
A_7	74	32
A_8	0	0
A_9	3	0
A_{10}	0	0
Mean Value	**10.7**	**4.3**
B_1	6	4
B_2	2	0
B_3	9	4
B_4	8	3
B_5	4	2
B_6	0	0
B_7	1	2
B_8	2	1
B_9	1	0
B_{10}	4	3
Mean Value	**3.7**	**1.9**
C_1	5	2
C_2	2	1
C_3	8	4
C_4	10	6
C_5	3	0
C_6	4	2
C_7	6	2
C_8	5	2
C_9	5	2
C_{10}	7	3
Mean Value	**5.5**	**2.5**
D_1	1	1
D_2	1	2
D_3	1	1
D_4	2	2
D_5	3	0
D_6	3	1
D_7	10	8
D_8	9	4
D_9	52	40
D_{10}	10	6
Mean Value	**9.2**	**6.5**

E_1	22	11
E_2	17	10
E_3	32	26
E_4	9	4
E_5	11	7
E_6	50	32
E_7	72	20
E_8	43	30
E_9	62	43
E_{10}	81	34
Mean Value	**39.9**	**21.7**

A, B, C, D and E - period of collection of sample (Days)
1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 - point of sample collection.

Table 3: The Number of Bacterial Isolated and the Percentage Occurrence.

Organisms	Period of isolation					Total	
	A	B	C	D	E	Percentage Occurrence	
Escherichia coli	3	5	9	5	10	32	32%
Enterobacter spp	2	3	4	3	6	18	18%
Klebsiella spp	1	2	4	2	5	14	14%
Bacillus spp	1	1	4	2	4	12	12%
Enterococcus spp	1	1	3	2	3	10	10%
Salmonella spp	0	1	2	1	2	6	6%
Pseudomonas spp	0	0	1	1	2	4	4%
Staphylococcus aureus	1	0	2	0	1	4	4%
TOTAL	9	13	29	16	33	100	100%

A, B, C, D and E - period of collection of sample (Days)

Table 4: Antibiotics Resistance Pattern of the Isolated Bacteria

Test Organism	AUG	NIT	CAZ	CRX	GEN	CTX	OFL	AMX	Phenotype of resistance pattern
Escherichia coli									
1	R	R	R	R	R	R	S	R	AUG, NIT, CAZ, CRX,GEN, CTX, AMX
2	R	R	R	R	I	R	S	R	AUG, NIT, CAZ, CRX,CTX, AMX
3	R	R	R	R	I	R	R	R	AUG, NIT, CAZ, CRX,CTX, OFL, AMX,
4	R	R	R	R	R	R	R	R	AUG, NIT, CAZ, CRX,GEN, CTX, OFL, AMX
5	R	R	R	R	R	R	S	R	AUG, NIT, CAZ, CRX,GEN, CTX, AMX
6	R	R	R	R	S	R	S	R	AUG, NIT, CAZ, CRX,CTX, AMX
7	R	R	R	R	R	R	S	R	AUG, NIT, CAZ, CRX,GEN, CTX, AMX
8	R	R	R	R	I	R	S	R	AUG, NIT, CAZ, CRX,CTX, AMX
9	R	R	R	R	R	R	R	R	AUG, NIT, CAZ, CRX,GEN, CTX, OFL, AMX
10	R	R	R	R	R	R	R	R	AUG, NIT, CAZ, CRX,GEN, CTX, OFL, AMX
11	R	R	R	R	R	R	S	R	AUG, NIT, CAZ, CRX,GEN,

									CTX, AMX
12	R	R	R	R	I	R	S	R	AUG, NIT, CAZ, CRX,CTX, AMX
13	R	R	R	R	R	R	S	R	AUG, NIT, CAZ, CRX,GEN, CTX, AMX
14	R	R	R	R	S	R	S	R	AUG, NIT, CAZ, CRX,CTX, AMX
15	R	R	R	R	I	R	R	R	AUG, NIT, CAZ, CRX,CTX, OFL, AMX
16	R	R	R	R	R	R	R	R	AUG, NIT, CAZ, CRX,GEN, CTX, OFL, AMX
17	R	R	R	R	R	R	S	R	AUG, NIT, CAZ, CRX,GEN, CTX, AMX
18	R	R	R	R	S	R	S	R	AUG, NIT, CAZ, CRX,CTX, AMX
19	R	R	R	R	R	R	S	R	AUG, NIT, CAZ, CRX,GEN, CTX, AMX
20	R	R	R	R	S	R	S	R	AUG, NIT, CAZ, CRX,CTX, AMX
21	R	R	R	R	S	R	R	R	AUG, NIT, CAZ, CRX,CTX, OFL, AMX
22	R	R	R	R	R	R	R	R	AUG, NIT, CAZ, CRX,GEN, CTX, OFL, AMX
23	R	R	R	R	R	R	S	R	AUG, NIT, CAZ, CRX,GEN, CTX, AMX
24	R	R	R	R	S	R	S	R	AUG, NIT, CAZ, CRX,CTX, AMX
25	R	R	R	R	R	R	S	R	AUG, NIT, CAZ, CRX,GEN, CTX, AMX
26	R	R	R	R	R	R	S	R	AUG, NIT, CAZ, CRX,GEN, CTX, AMX
27	R	R	R	R	R	R	R	R	AUG, NIT, CAZ, CRX,GEN, CTX, OFL, AMX
28	R	R	R	R	R	R	R	R	AUG, NIT, CAZ, CRX,GEN, CTX, OFL, AMX
29	R	R	R	R	R	R	S	R	AUG, NIT, CAZ, CRX,GEN, CTX, AMX
30	R	R	R	R	I	R	S	R	AUG, NIT, CAZ, CRX,CTX, AMX
31	R	R	R	R	R	R	S	R	AUG, NIT, CAZ, CRX,GEN, CTX, AMX
32	R	R	R	R	S	R	S	R	AUG, NIT, CAZ, CRX, CTX, AMX
% resistant to antibiotic	100	100	100	100	59	100	31	100	
Enterobacter spp									
1	R	R	R	R	I	R	S	R	AUG, NIT, CAZ, CRX, CTX, AMX
2	R	R	R	R	S	R	S	R	AUG, NIT, CAZ, CRX, CTX, AMX
3	R	R	R	R	S	R	S	R	AUG, NIT, CAZ, CRX, CTX, AMX
4	R	R	R	R	R	R	S	R	AUG, NIT, CAZ, CRX, GEN, CTX, AMX
5	R	R	R	R	R	R	R	R	AUG, NIT, CAZ, CRX, GEN,

									CTX, OFL, AMX
6	R	R	R	R	I	R	I	R	AUG, NIT, CAZ, CRX, CTX, AMX
7	R	R	R	R	S	R	S	R	AUG, NIT, CAZ, CRX, CTX, AMX
8	R	R	R	R	R	R	R	R	AUG, NIT, CAZ, CRX, GEN, CTX, OFL, AMX
9	R	R	R	R	S	R	S	R	AUG, NIT, CAZ, CRX, CTX, AMX
10	R	R	R	R	S	R	S	R	AUG, NIT, CAZ, CRX, CTX, AMX
11	R	R	R	R	S	R	S	R	AUG, NIT, CAZ, CRX, CTX, AMX
12	R	R	R	R	R	R	S	R	AUG, NIT, CAZ, CRX, GEN, CTX, AMX
13	R	R	R	R	R	R	I	R	AUG, NIT, CAZ, CRX, GEN, CTX, AMX
14	R	R	R	R	R	R	S	R	AUG, NIT, CAZ, CRX, GEN, CTX, AMX
15	R	R	R	R	S	R	S	R	AUG, NIT, CAZ, CRX, CTX, AMX
16	R	R	R	R	R	R	S	R	AUG, NIT, CAZ, CRX, GEN, CTX, AMX
17	R	R	R	R	S	R	S	R	AUG, NIT, CAZ, CRX, CTX, AMX
18	R	R	R	R	R	R	S	R	AUG, NIT, CAZ, CRX, GEN, CTX, AMX
% resistant to antibiotic	100	100	100	100	44	100	11	100	
Salmonella Spp									
1	R	R	R	R	S	R	S	R	AUG, NIT, CAZ, CRX, CTX, AMX
2	R	R	R	R	R	R	S	R	AUG, NIT, CAZ, CRX, GEN, CTX, AMX
3	R	R	R	R	R	R	R	R	AUG, NIT, CAZ, CRX, GEN, CTX, OFL, AMX
4	R	R	R	R	R	R	R	R	AUG, NIT, CAZ, CRX, GEN, CTX, OFL, AMX
5	R	R	R	R	I	R	I	R	AUG, NIT, CAZ, CRX, CTX, AMX
6	R	R	R	R	I	R	I	R	AUG, NIT, CAZ, CRX, CTX, AMX
% resistant to antibiotic	100	100	100	100	50	100	33	100	
Klebsiella spp									
1	R	R	R	R	S	R	S	R	AUG, NIT, CAZ, CRX, CTX, AMX
2	R	R	R	R	I	R	I	R	AUG, NIT, CAZ, CRX, CTX, AMX
3	R	R	R	R	S	S	S	R	AUG, NIT, CAZ, CRX, AMX
4	R	R	R	R	R	R	R	R	AUG, NIT, CAZ, CRX, GEN, CTX, OFI, AMX
5	R	R	R	R	S	S	I	R	AUG, NIT, CAZ, CRX, AMX
6	R	R	R	R	R	R	S	R	AUG, NIT, CAZ, CRX, GEN,

									CTX, AMX
7	R	R	R	R	S	R	R	R	AUG, NIT, CAZ, CRX, CTX,OFL, AMX
8	R	R	R	R	R	R	R	R	AUG, NIT, CAZ, CRX, GEN, CTX, OFL, AMX
9	R	R	R	R	S	R	S	R	AUG, NIT, CAZ, CRX, CTX, AMX
10	R	R	R	R	R	R	R	R	AUG, NIT, CAZ, CRX, GEN, CTX, OFI, AMX
11	R	R	R	R	S	S	S	R	AUG, NIT, CAZ, CRX, AMX
12	R	R	R	R	R	R	R	R	AUG, NIT, CAZ, CRX, GEN, CTX, OFL, AMX
13	R	R	R	R	S	S	I	R	AUG, NIT, CAZ, CRX, AMX
14	R	R	R	R	R	R	S	R	AUG, NIT, CAZ, CRX, GEN, CTX, AMX
% resistance to antibiotic	100	100	100	100	43	71	36	100	
Pseudomonas spp									
1	R	R	R	R	R	R	I	R	AUG, NIT, CAZ, CRX, GEN, CTX, AMX
2	R	R	R	R	S	R	I	R	AUG, NIT, CAZ, CRX, CTX, AMX
3	R	R	R	R	R	R	S	R	AUG, NIT, CAZ, CRX, GEN, CTX, AMX
4	R	R	R	R	R	R	S	R	AUG, NIT, CAZ, CRX, GEN, CTX, AMX
% resistance to antibiotic	100	100	100	100	75	100	100	100	

Isolates	**Antibiotics**								**Phenotype of resistance pattern**
	AUG	**LIN**	**CAZ**	**CRX**	**GEN**	**OXC**	**OFL**	**OXA**	
Staphylococus aureus									
1	R	R	S	S	S	R	S	R	AUG, LIN, COX, OXA
2	R	R	S	I	S	R	I	R	AUG, LIN, COX, OXA
3	R	R	I	I	S	R	S	R	AUG, LIN, COX, OXA
4	R	R	S	S	S	R	S	R	AUG, LIN, COX, OXA
% resistance to antibiotic	100	100	0	0	0	100	0	100	
Bacillus spp.									
1	R	R	R	R	S	R	S	R	AUG, LIN, CAZ, CRX, COX, OXA
2	R	R	R	R	S	R	S	R	AUG, LIN, CAZ, CRX, COX, OXA
3	R	R	R	R	S	R	S	R	AUG, LIN, CAZ, CRX, COX, OXA
4	R	R	R	R	S	R	S	R	AUG, LIN, CAZ, CRX, COX, OXA
5	R	R	R	R	S	R	S	R	AUG, LIN, CAZ, CRX, COX, OXA
6	R	R	R	R	S	R	S	R	AUG, LIN, CAZ, CRX, COX,

									OXA
7	R	R	R	R	S	R	S	R	AUG, LIN, CAZ, CRX, COX, OXA
8	R	R	R	R	S	R	S	R	AUG, LIN, CAZ, CRX, COX, OXA
9	R	R	R	R	S	R	S	R	AUG, LIN, CAZ, CRX, COX, OXA
10	R	R	R	R	S	R	S	R	AUG, LIN, CAZ, CRX, COX, OXA
11	R	R	R	R	S	R	S	R	AUG, LIN, CAZ, CRX, COX, OXA
12	R	R	R	R	S	R	S	R	AUG, LIN, CAZ, CRX, COX, OXA
% of resistance to antibiotic	100	100	100	100	0	100	0	100	
Enterococcus spp									
1	R	R	S	R	S	R	S	R	AUG, LIN, CRX, COX, OXA
2	R	R	I	R	S	R	S	R	AUG, LIN, CRX, COX, OXA
3	R	R	S	R	R	R	S	R	AUG, LIN, CRX, COX, OXA
4	R	R	R	R	R	R	S	R	AUG, LIN, CAZ, CRX, OXC, OXA
5	R	R	S	R	S	R	R	R	AUG, LIN, CRX, OXC, OFL, OXA
6	R	R	S	R	S	R	S	R	AUG, LIN, CRX, COX, OXA
7	R	R	I	R	R	R	R	R	AUG, LIN, CRX, GEN, COX, OFL, OXA
8	R	R	R	R	R	R	S	R	AUG, LIN, CAZ, CRX, GEN, COX
9	R	R	I	R	R	R	S	R	AUG, LIN, CRX, GEN, COX, OXA
10	R	R	S	R	S	R	S	R	AUG, LIN, CRX, COX, OXA
% resistance to antibiotic	100	100	20	100	50	100	20	100	

CAZ–Cefotaxime, CRX – Cefuroxime, GEN- Gentamycin, OFL-Ofloxacin, AUG-Augmentin, NIT-Nitrofurantoin, CTX-Ceftazidime, AMX-Amoxicillin, LIN-Lincomycin, OXA-Oxacilin and COX-Cloxacilin.
R – Resistant S – Sensitive I – Intermediate

Table 5: Result of the Heavy Metal Analysis in the leachate Samples

Parameters (mg/L)	**A**	**B**	**C**	**D**	**E**
	ND	0.01	0.002	0.01	0.01
	0.02	ND	ND	0.001	0.02
Zinc (Zn)	ND	ND	ND	0.02	0.01
	ND	0.001	0.001	0.01	0.02
	0.00l	0.02	0.02	0.02	0.02
	ND	ND	ND	0.001	ND
	ND	ND	ND	0.001	0.001
Lead (Pb)	ND	ND	ND	0.01	ND
	ND	ND	ND	0.001	0.001
	ND	ND	ND	0.002	0.002
	ND	ND	0.001	0.01	0.01
	ND	ND	0.001	ND	0.001
Copper(Cu)	ND	ND	ND	0.01	ND
	0.00l	0.01	ND	0.02	0.01
	0.01	ND	0.001	0.01	0.01

	ND	0.01	ND	0.01	ND
	0.01	0.001	ND	ND	0.01
Cobalt (Co)	0.02	ND	ND	ND	0.01
	0.01	0.01	ND	ND	ND
	ND	ND	ND	ND	0.01
	ND	ND	ND	ND	ND
	ND	ND	ND	ND	ND
Mercury (Hg)	ND	ND	ND	ND	ND
	ND	ND	ND	ND	ND
	ND	ND	ND	ND	ND

A, B, C, D, E - period of sample collection (Days)
ND - not detected

Table 6: Activity Concentrations (Bq kg^{-1}), Dose Rates (D_{out} nGy h^{-1}) and Annual effective Dose (E_{air} mSv y^{-1})

Sampling Location	^{40}K	^{226}Ra(^{238}U)	^{208}Tl(^{232}Th)	D_{out}	E_{air}
1	908±70	39±3	8±1	64±9	0.08
2	769±58	44±3	12±3	61±11	0.08
3	804±69	41±3	12±3	61±9	0.08
4	1209±71	34±3	10±2	65±8	0.09
5	1180±75	19±2	9±2	62±10	0.08
Avg	**974±67**	**35±3**	**10±2**	**63±9**	**0.08**

Duncan Multiple Range Test (DMRT) of the Total Bacterial and Coliform Count

Collection period	TBC Dilution Factor		TCC Dilution Factor	
	10^7	10^8	10^7	10^8
A	17.60±21.84^{b}	7.30±9.36^{c}	10.70±22.40^{b}	4.30±9.83^{b}
B	19.50±19.31^{b}	12.10±12.67^{bc}	3.70±3.09^{b}	1.90±1.59^{b}
C	40.00±20.28^{b}	26.50±16.93^{ab}	5.50±2.37^{b}	2.40±1.65^{b}
D	33.00±25.68^{b}	21.00±19.72^{abc}	9.20±15.50^{b}	6.50±12.04^{b}
E	70.60±43.79^{a}	33.30±22.49^{a}	39.9±25.86^{a}	21.70±13.26^{a}

Duncan Multiple Range Test (DMRT) of the Total Bacterial and Coliform Count

Sampling Point	TBC Dilution Factor		TCC Dilution Factor	
	10^7	10^8	10^7	10^8
Point 1	28.00±17.89^{a}	16.20±10.99^{a}	8.20±8.04^{a}	4.20±3.96^{a}
Point 2	16.20±5.07^{a}	8.80±4.77^{a}	5.00±6.75^{a}	3.00±4.00^{a}
Point 3	31.40±18.39^{a}	13.80±7.56^{a}	10.60±12.42^{a}	7.20±10.62^{a}
Point 4	26.80±11.65^{a}	19.20±8.79^{a}	7.40±3.13^{a}	3.80±1.49^{a}
Point 5	22.20±17.34^{a}	14.00±16.40^{a}	4.80±3.49^{a}	1.80±3.03^{a}
Point 6	38.40±31.92^{a}	19.20±15.67^{a}	12.60±21.02^{a}	7.20±13.88^{a}
Point 7	48.40±28.22^{a}	21.60±10.26^{a}	32.60±37.02^{a}	12.80±13.01^{a}
Point 8	38.20±38.41^{a}	21.60±19.31^{a}	11.80±17.77^{a}	7.40±12.72^{a}
Point 9	60.00±64.31^{a}	31.60±32.21^{a}	24.60±29.82^{a}	17.00±22.41^{a}
Point 10	51.80±49.95^{a}	34.40±36.26^{a}	20.40±34.08^{a}	9.20±14.02^{a}

TBC - Total Bacterial Count
TCC - Total Coliform Count

CONCLUSION AND RECOMMENDATION

This study has revealed that serious health hazards could result from the contamination of aquatic environment by leachate. From the research work, it is evident that the microbial load of the leachate is high comprising mainly of coliforms. There is need for proper treatment of effluents before they are been discharge into the waterbodies to prevent the risk of getting infected by waterborne diseases. Sewage and refuse should not be dumped into the stream water around the landfill site in order not to increase the nutrient availability of the water which will allow the growth of organisms in the water. There is urgent need for awareness to be created about the present situation of the leachate and how it can affect the environment to alert the communities living around the area on the needs for treatment of the stream around the landfill before they can be used for drinking and other domestic uses and also to suggest possible solutions to problems that may arise from these resistance strains that could invade the communities from drinking the water. Finally, the results from this study also challenge the scientists on the need for more or development of new antibiotics to combat the infections caused by these resistance strains.

The activity concentrations of naturally occurring radionuclides, absorbed dose rates and annual effective dose have been determined. The average values of all these radiological parameters are within acceptable limit. This indicates that soil samples and site are safe from radiological burden. This study has provided helpful data for future assessment in case of gross contamination of the site in the future.

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